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Preliminary communication

VIABILITY OF BIFIDOBACTERIA IN SOFT-FROZEN ICE CREAM SUPPLEMENTED WITH A *SACCHAROMYCES CEREVISIAE* CELL WALL PRODUCT

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The purpose of this research was to monitor the changes during storage in survival of bifidobacteria in a soft-frozen ice cream supplemented with a yeast cell wall-based product claimed to contribute to the functioning of the immune system. An ice cream mix was prepared and pasteurised. After overnight aging at 4 °C, it was inoculated with *Bifidobacterium animalis* subsp. *lactis* Bb-12. Two batches of the mix were supplemented with a commercial *Saccharomyces cerevisiae* cell wall product at 2.0% and 4.0% (w/w), whereas a third batch was left unsupplemented and served as control. The final mixes were frozen, and the three products were stored at –13 °C for 7 days. The ice creams contained viable bifidobacteria cells at levels exceeding 10^6 CFU g^{-1} throughout the storage period. Although the yeast supplement decreased the loss of viability of bifidobacteria during frozen storage of ice creams, it imparted a slightly bitter off-flavour to the samples and it also negatively influenced the original white colour of the product, thereby necessitating further work to develop flavoured varieties of the *Saccharomyces* cell wall-containing synbiotic ice cream.

Keywords: *Bifidobacterium*, *Saccharomyces*, ice cream, probiotic, prebiotic, synbiotic

Over the past few decades, numerous strains of *Lactobacillus* and *Bifidobacterium* species have attracted attention as probiotic microorganisms (Schiffrin et al., 1995; Pereira et al., 2010; Matias et al., 2016) and, as a result, have been incorporated into hundreds of nutritionally functional foods and food supplements worldwide (Süle et al., 2014; Varga et al. 2014b). The capacity of probiotic microbes to exert positive effects on the consumer depends on the number of viable cells reaching the large intestine (Varga et al., 2014a). According to the Hungarian Food Code, Codex Alimentarius Hungaricus, health benefits can only be expected if probiotics are present in foods at concentrations exceeding 10^6 CFU g^{-1} at the time of consumption (Codex Alimentarius Hungaricus Commission, 2004). Various reports have shown, however, that many commercial probiotic products contain viable cells well below minimum regulatory limits (Kolaček et al., 2017).

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The growth and viability of probiotic organisms may considerably be improved by the addition of specific substrates known as prebiotics (Gibson & Roberfroid, 1995). The major groups of substances having prebiotic potential include: galacto- and fructo-oligosaccharides, inulin, lactulose, resistant starch, pectin, human milk oligosaccharides, xyloglucan and yeast cell wall polysaccharides (Hutkins et al., 2016). Prebiotics can readily be combined with probiotics to result in synbiotics (Silva et al., 2017). Such products have the properties of health-promoting functional foods affecting important functions of the human body in a positive and targeted manner (Gibson & Roberfroid, 1995; Schrezenmeir & De Vrese, 2001).

Ice cream is the most popular frozen dairy dessert in many parts of the world. It is manufactured globally in a wide variety of shapes and flavours, and can be categorised as hard-frozen and soft-frozen products, the latter being mostly prepared at the site of purchase and consumption (Goff, 2011). The nutritional value of ice cream may be enhanced through supplementation with health-promoting ingredients, including minerals, vitamins, prebiotics and probiotic cultures. Synbiotic ice cream is an effective and relatively innovative vehicle for delivering beneficial lactobacilli and bifidobacteria to the human gastrointestinal tract (Cruz et al., 2009; Mohammadi et al., 2011).

The objective of this study was to monitor the changes during storage in survival of *Bifidobacterium animalis* subsp. *lactis* Bb-12 in a soft-frozen artisanal ice cream supplemented with a novel yeast cell wall-based product resulting from the autolysis of *Saccharomyces cerevisiae*. The commercial preparation, which is a good source of β-(1→3),(1→6)-glucans, is claimed by the manufacturer to contribute to the proper functioning of the immune system. To our knowledge, this is the first research aimed at developing and evaluating a yeast cell wall-containing synbiotic ice cream.

1. Materials and methods

1.1. Production and frozen storage of ice creams

The basic ice cream mix containing 1000 g of milk with 3.5% fat, 40 g of butter with 82% fat, 220 g of sucrose, 30 g of dextrose and 3 g of sodium alginate was prepared and heat-treated in a Pastomaster RTL pasteurising machine (Carpigiani, Anzola Emilia, Italy), with the latter process being carried out at 85 °C for 20 s. After 24 h of aging at 4 °C, the mix was inoculated with a *B. animalis* subsp. *lactis* Bb-12 lyophilised direct vat set culture (Chr. Hansen, Hørsholm, Denmark) to provide an initial probiotic cell density of approximately 10^8 CFU g^{-1}. The first two batches of mix were also supplemented with Lynside Wall Basic (Lesaffre, Maisons Alfort, France), a commercial yeast cell wall product prepared from *Saccharomyces cerevisiae*, at 2.0% and 4.0% (w/w), respectively, whereas the third batch was left unsupplemented and served as control. The final ice cream mixes were frozen in a Labotronic 15-45 RTX batch freezer (Carpigiani) for 6 min. The three products were each separated into nine fractions that were transferred in sterile tightly capped centrifuge tubes (50 ml; Greiner Bio-One Hungary, Mosonmagyaróvár, Hungary) and stored at –13 °C for 7 days.

1.2. Microbiological analysis

Three tubes of all three products were taken at each sampling time, i.e., following 0, 3 and 7 days of frozen storage. Samples were aseptically removed from centrifuge tubes and diluted by mixing 10 g with 90 ml of peptone saline water containing 0.1% casein peptone and 0.85% sodium chloride. Further dilutions were made as required. The pour-plate technique with De Man–Rogosa–Sharpe (MRS) agar (Merck, Darmstadt, Germany) was used for the enumeration of *B. animalis* subsp. *lactis* Bb-12. The plates were incubated at 37 °C for 72 h. Anaerobic conditions were generated using anaerobic culture jars (2.5 l) and AnaeroGen AN 25 sachets (Oxoid, Basingstoke, UK). The counts were expressed as $\log_{10}$ CFU g^{-1}. The bifidobacteria colonies identified were irregularly shaped or lenticular, and were corroborated by observation under a transmitted light microscope (KF 2 ICS; Carl Zeiss Microscopy, Jena, Germany). *Enterobacteriaceae*, *Escherichia coli* and coagulase-positive staphylococci counts were enumerated and the presence or absence of *Salmonella* spp. and *Listeria monocytogenes* were detected according to international standard procedures (ISO, 1999, 2005, 2017a,b,c).

1.3. Determination of pH value

The pH value of samples was measured with a Jenway 3510 pH-meter and combined glass electrode (Keison Products, Chelmsford, UK) standardised with pH 7.00 and 4.00 standard buffer solutions (Merck).

1.4. Statistical analysis

The results were subjected to ANOVA using the general linear model procedure of STATISTICA data analysis software system (version 9.0; StatSoft, Tulsa, OK). Significant differences among the $\log_{10}$ CFU g^{-1} or pH means were determined by using Duncan's multiple comparison test at $P<0.05$ (StatSoft Inc.).

2. Results and discussion

Table 1 shows the changes in viability of *B. animalis* subsp. *lactis* Bb-12 during frozen storage of ice creams at –13 °C. Mean bifidobacteria counts exceeded 7.50 $\log_{10}$ CFU g^{-1} in the control ice cream at the start of the storage period. The value of 7.52 $\log_{10}$ CFU g^{-1} was significantly higher ($P<0.05$) than those determined initially in the other two products containing yeast cell wall. This observation may be explained by the fact that, as was mentioned in subsection 1.1., the three basic ice cream mixes were not inoculated with exactly the same levels of probiotic cells and, as a result, the viable counts of bifidobacteria happened to differ on day 0. With the progress of storage time, there was a significant decline ($P<0.05$) in viable numbers of *B. animalis* subsp. *lactis* Bb-12 in all three ice cream formulations. Although the control product had the lowest bifidobacteria survival rate on day 7, the final viable counts did not largely differ among treatments. Following one week of frozen storage, more than one third of the initial probiotic population was viable and culturable in the ice creams supplemented with *S. cerevisiae* cell wall, whereas this was only true for approximately one fifth of bifidobacteria in the control product.

Table 1. Effect of a *Saccharomyces cerevisiae* cell wall product on survival of *Bifidobacterium animalis* subsp. *lactis* Bb-12 in soft-frozen ice cream during storage at –13 °C

Storage time (day)	Ice cream containing yeast cell wall supplement at					
	0.0% (Control)		2.0% (w/w)		4.0% (w/w)	
	Log_{10} CFU g^{-1}*	%	Log_{10} CFU g^{-1}*	%	Log_{10} CFU g^{-1}*	%
0	$7.52\pm0.06^{A,a}$	100.0	$7.29\pm0.03^{A,b}$	100.0	$7.31\pm0.03^{A,b}$	100.0
3	$7.11\pm0.04^{B,b}$	39.4	$7.20\pm0.03^{B,a}$	90.0	$7.05\pm0.09^{B,b}$	52.4
7	$6.85\pm0.01^{C,a}$	21.2	$6.83\pm0.01^{C,b}$	34.0	$6.86\pm0.02^{C,a}$	34.3

*: Values are means ± SD, based on three observations.
^{A,B,C}: Means within a column with different uppercase superscripts differ ($P<0.05$).
^{a,b}: Means within a row with different lowercase superscripts differ ($P<0.05$).

As shown in Table 1, all the samples tested in the present study contained *B. animalis* subsp. *lactis* Bb-12 at levels well exceeding the regulatory minimum of 10^6 CFU g^{-1}. This finding is consistent with those of previous reports demonstrating that bifidobacteria can survive and remain above 10^6 CFU g^{-1} in probiotic and synbiotic ice creams stored at –18 °C for up to 3 months (Rezaei et al., 2014; Matias et al., 2016; Cruxen et al., 2017). In another study, the viable cell counts of bifidobacteria in a fermented probiotic ice cream decreased from 8.70 to 7.00 log_{10} CFU ml^{-1} following 17 weeks of storage at –29 °C. It is worth mentioning that after the first week of frozen storage, half (i.e., 8.40 log_{10} CFU ml^{-1}) of the initial *B. bifidum* 10LF population was culturable (Hekmat & McMahon, 1992). This survival rate (50%) is higher than those observed in our trials (21–34%); however, the conditions used in the two studies were not identical.

Our soft-frozen ice cream samples were not only tested for viability of beneficial bifidobacteria, but also for the presence of pathogenic and spoilage organisms. All three product formulations were found to be microbiologically safe for human consumption, because they were free from *Salmonella* spp. and *L. monocytogenes*, and contained no *E. coli* and coagulase-positive staphylococci, indicating the high standards of sanitation during manufacturing and packaging of ice creams. Di Criscio and co-workers (2010) and Matias and co-workers (2016) also found very low or nondetectable levels of enterobacteria, coliforms and *E. coli* in synbiotic ice creams stored at –20 °C for 16 weeks and –18 °C for 12 weeks, respectively.

As illustrated in Table 2, supplementation with at least 2.0% of *Saccharomyces* cell wall product resulted in a significant decrease ($P<0.05$) in the pH of ice cream. This observation is in agreement with that of Rezaei and co-workers (2014), who reported that a synbiotic frozen yogurt containing 2.0% of inulin had significantly lower ($P<0.05$) pH values compared to the control product. In contrast, the duration of frozen storage had no consistent effect on pH values (Table 2). Similarly, a recent study by Di Criscio and co-workers (2010) has also indicated that the acidity values of both control and synbiotic ice creams remain unchanged during frozen storage at –20 °C.

The three ice cream formulations were finally subjected to sensory evaluation by untrained panellists (data not shown). The results indicated that the yeast cell wall supplement imparted a slightly bitter off-flavour to the samples and it also negatively influenced the original white colour of the product, thereby necessitating further work to develop flavoured varieties of the *Saccharomyces* cell wall-containing soft-frozen synbiotic ice cream. Similarly, in an another study by Di Criscio and co-workers (2010), the colour of synbiotic ice creams

containing probiotic lactobacilli and inulin was found to be poorer, i.e., more opaque, than that of the control product. Several authors point out that it is difficult but not impossible to produce organoleptically acceptable synbiotic ice creams (Mohammadi et al., 2011). For instance, sensory properties can be improved by supplementation with various fruits because of their sweet and sour taste (Karaman et al., 2014; Cruxen et al., 2017).

Table 2. Effect of a *Saccharomyces cerevisiae* cell wall product on the pH value* of soft-frozen ice cream during storage at –13 °C

Storage time (day)	Ice cream containing yeast cell wall supplement at		
	0.0% (Control)	2.0% (w/w)	4.0% (w/w)
0	$5.98\pm0.04^{A,a}$	$5.56\pm0.02^{C,b}$	$5.31\pm0.01^{B,c}$
3	$6.10\pm0.01^{B,a}$	$5.68\pm0.01^{A,b}$	$5.43\pm0.01^{A,c}$
7	$5.91\pm0.01^{A,a}$	$5.61\pm0.01^{B,b}$	$5.44\pm0.01^{A,c}$

*: Values are means ± SD, based on three observations.
^{A,B,C}: Means within a column with different uppercase superscripts differ ($P<0.05$).
^{a,b,c}: Means within a row with different lowercase superscripts differ ($P<0.05$).

3. Conclusions

Even though currently there are no synbiotic soft-frozen ice creams on the market in Hungary, it is technologically feasible to manufacture such products that comply with relevant food standards and regulations. The artisanal ice cream formulations developed in this study were found to contain viable bifidobacteria cells at concentrations exceeding 10^6 CFU g^{-1} for at least 7 days of frozen storage at –13 °C, thus meeting national regulatory requirements for probiotic dairy foods. The *S. cerevisiae* cell wall supplement tested decreased the loss of viability of *B. animalis* subsp. *lactis* Bb-12 during frozen storage of ice creams; however, it negatively affected the sensory properties of the final products. For this reason, further product development activities and economic calculations are needed to determine whether flavoured synbiotic ice creams can profitably be manufactured and commercialised. If so, artisanal ice cream makers would be well positioned to successfully compete with industrial producers. In addition, an increase in consumption of synbiotic ice creams might beneficially influence the health status of the general population.

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